SIDDHA PAPERS
A PEER REVIEWED ONLINE JOURNAL
ISSN: 0974-2522

Siddha Papers 2019 (14) (1)
RESEARCH ARTICLE
https://www.siddhapapers.com

Anticancer activity of *Vettukaaya Poondu* (*Tridax procumbens)* as evidenced by Cytotoxic assay

T. V. Sundhari[1], R. Soundarya[1], D. Jansirani[1], P. Lavanya[1], Thomas M Walter[2].

[1]Third year BSMS, Government Siddha Medical College, Palayamkottai, Tirunelveli, Tamil Nadu, ragavi240499@gmail.com

[2]Lecturer, Government Siddha Medical College, Palayamkottai, Tirunelveli, Tamil Nadu, siddhawalter@gmail.com

ABSTRACT

Indian traditional medicine is provenance of many herbs which are mostly left unnoticed. Many anti-cancerous plants and minerals are mentioned in the classical Siddha Literatures which have a wide range of benefits. *Vettukaaya poondu* (*Tridax procumbens)* is one such weed available throughout the continent. In many places around Tamil Nadu, it was also called as Thatha Thalai chedi. From this, the fresh juice of the plant has widely used for treating ulcers. This plant is present throughout India and is employed as indigenous folklore medicine for variety of ailments. This is an analysis to fortify the knowledge of rich traditional practices followed since many years .*Tridax procumbens* of *Asteraceae* is mostly used for treating uncontrolled bleeding wounds, ulcers and cuts. Turmeric (*Curcuma longa)* belongs to the family *Zingiberaceae* is a major part of the siddha system and has recommended turmeric for medicine. It is used as an antiseptic medicine from ancient time onwards. Root part (Rhizome) of the plantare widely used by different tribal communities as turmeric have been shown to have wide spectrum of biological actions, which include anti-inflammatory, anti-cancerous, anti-diabetic, analgesic, anti bacterial, anti fungal, anti-protozoal, anti ulcer, hypocholesteremic activities and many more medicinal values. In this research study, carried out the *in-vitro* cytotoxic assay on A549 human lung carcinoma cell lines. The *In vitro* studies show significant inhibition for carcinoma cells. Thus further more clinical studies have to be carried out in large scale to provide further clinical viability.

KEYWORDS

Vettukaaya poondu, *Tridax procumbens*, *Curcuma longa*, Anti cancer, cytotoxic assay, in vitro assay.

INTRODUCTION

The plant kingdom harbors an inexhaustible source of active ingredients for the valuable treatment of diseases . Various studies have identified compounds from herbal plants that are effective antibiotics. Medicinal herbs play an important role in primary health care system among rural population since synthetic anti cancer remedies are beyond the reach of common man because of the cost factor. Phytochemicals derived from traditional medicinal plants have been found to possess anti cancer and chemoprotective effects. They provide nutrition and reduce the side effects of conventional cancer therapy due to effective anti oxidant activity. The in vitro MTT assay is used for the determination of cell proliferation and cytotoxicity of *Tridax procumbens* in combination with *Curcuma longa* against cancerous activity.

Tridax procumbens (coat buttons) is a common medicinal herb called kinatruppasan or vettukaaya poondu in Tamil belonging to family *Asteraceae*. The plant is native of tropical America and naturalized in tropical Africa, Asia, Australia and India. *Curcuma longa* of *Zingiberaceae* is a perennial rhizomatous, herbaceous plant native to the Indian sub continent and Southeast Asia .The greatest diversity of this species in India is at around 40 to 45 species.

Chemical Constituents

The flavanoid procumbenetin has been isolated from the aerial parts of *Tridax procumbens* . Other chemical compounds isolated from the plant include alkyl esters , sterol , pentacyclic triterpenes , fatty acids and polysaccharides.

Phytochemical components of turmeric include diarycheptanoids , a class including numerous curcuminoids such as curcumin, demethoxycurcumin and bisdemethoxycurcumin . Curcumin constitutes upto 3.14% of assayed commercial samples of turmeric powder . Some 34 essential oils are present in turmeric among which turmerone , germacrone , atlantone and zingiberene are major constituents.

MATERIALS AND METHODS

The formula for this preparation ‘Vettukaya poondu was selected .The raw drugs were collected from Tirunelveli district . After that the raw drugs were subjected to proper purification to remove all impurities . The purified ingredients are allowed to dry in sunshade for four days and then well grinded to powder form .This sample was subjected to cytotoxic studies.

Collection of plants

Vettukaaya poondu (*Tridax procumbens)* is collected in moist places beside pond and crop fields in village of Palayamkottai, Tirunelveli district. *Curcuma longa* is collected.

Purification of plants

The leaves of *Tridax* are used from the collected plants. Then the leaves are cleaned with water to remove the impurities. It is dried in sunshade for 4 days. *Curcuma longa* is also cleaned to remove the impurities present in it.

Preparation method

We grind the leaves of *Tridax* first and kept the powder in one container and then grind the *Curcuma* to powdered form. Finally we combined the powder of *Tridax* and the powder of *Curcuma* in the ratio of 2:1. Then it is stored in air tight container.

Requirement of the Study

To determine the Cytotoxicity Study on A549 Cell line with 5 different concentrations (25, 50,100,200, 400uG/mL) were used.

Reagents and Chemicals required

These are the listed chemicals used for the study which are Cell lines:A549-Human Lung Carcinoma Cell line (From NCCS, Pune), Cell culture medium: DMEM- High Glucose - (#AL111, Himedia), Adjustable multichannel pipettes and a pipettor (Benchtop, USA),Fetal Bovine Serum (#RM10432, Himedia), MTT Reagent (5 mg/ml) (# 4060 Himedia), DMSO (#PHR1309, Sigma), Camptothecin (#C9911, Sigma), D-PBS (#TL1006, Himedia)

EQUIPMENTS

1. Centrifuge (Remi: R-8^{o}C).
2. Pipettes: 2-10µl, 10-100µl, and 100-1000µl.
3. Inverted microscope (Biolink)
4. 37°C incubator with humidified atmosphere of 5% CO_2 (Healforce, China)

MTT assay

MTT assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow colored water soluble tetrazolium dye MTT to

formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple color, the intensity of which is proportional to the number of viable cells and can be measured spectrophotometrically at 570nm.(Alley, M. C et al., 1986, Mosmann et al., 1983).Extracellular reducing components such as ascorbic acid, cholesterol, alpha-tocopherol, dithiothreitol present in the culture media may reduce the MTT to formazan. To account for this reduction, it is important to use the same medium in control as well as test wells.

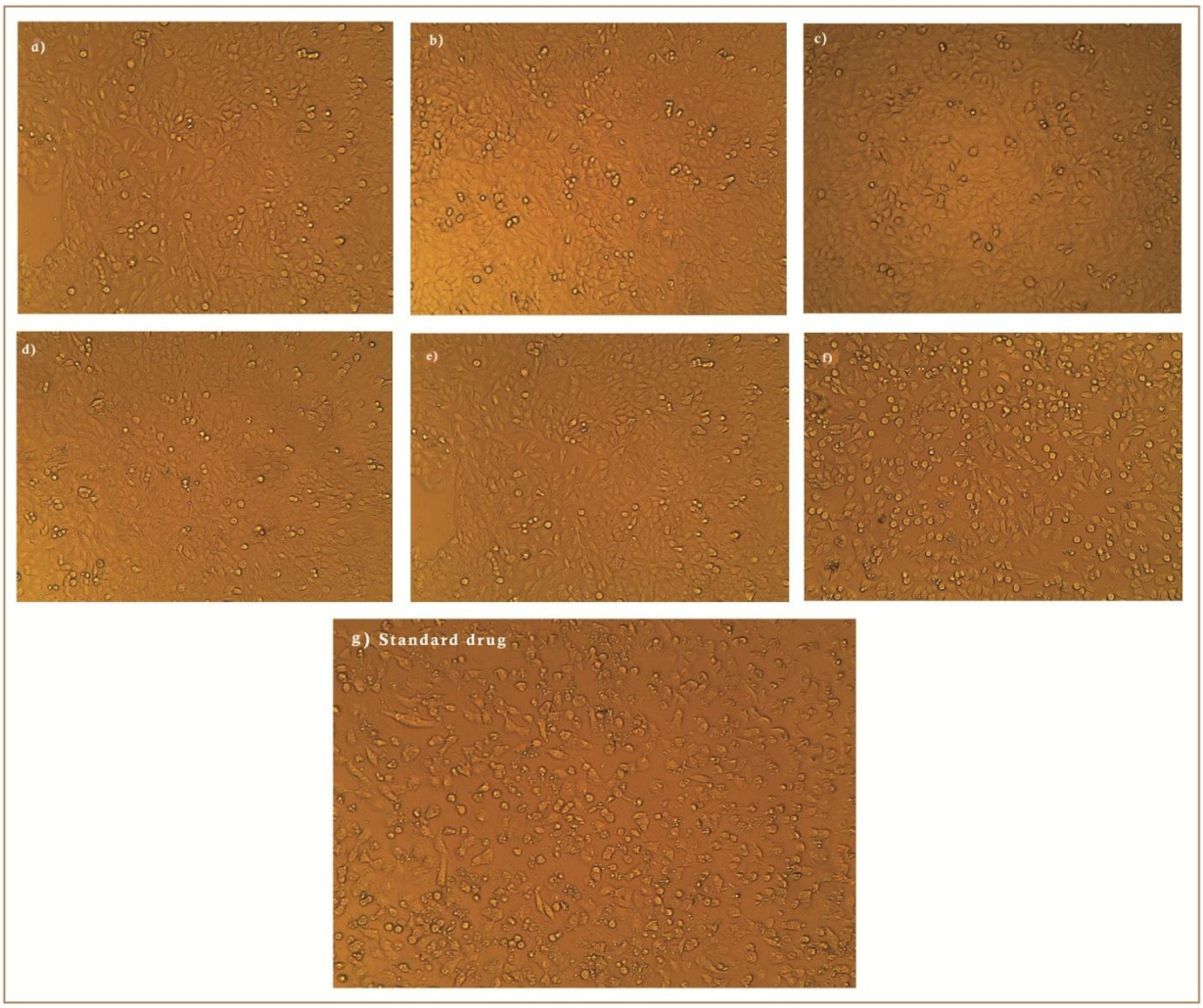
STEPS FOLLOWED

The Seed 200µl cell suspension in a 96-well plate at required cell density (20,000 cells per well), without the test agent. Allow the cells to grow for about 24 hours. Added the appropriate concentrations of the test agent. Incubate the plate for 24 hrs at 37°C in a 5% CO2 atmosphere. After the incubation period, takeout the plates from incubator, and remove spent media and add MTT reagent to a final concentration of 0.5mg/mL of total volume. Wrap the plate with aluminium foil to avoid exposure to light. Return the plates to the incubator and incubate for 3 hours. (Note: Incubation time varies for different cell lines. Within one experiment, incubation time should be kept constant while making comparisons.) Remove the MTT reagent and then add 100 µl of solubilisation solution (DMSO). Gentle stirring in a gyratory shaker will enhance dissolution. Occasionally, pipetting up and down may be required to completely dissolve the MTT formazan crystals especially in dense cultures. Read the absorbance on a spectrophotometer or an ELISA reader at 570nm and 630nm used as reference wavelength. **The IC50 value** was determined by using linear regression equation i.e. Y =Mx+C. Here, Y = 50, M and C values were derived from the viability graph.

RESULTS

In this study, 4Test Compounds are evaluated to check the Cytotoxicity Study on the 1cell line namely, A549. The combination of *Tridax and Curcuma* is treated in the concentration of 25, 50,100,200,400uG/ml with standard 10Um Campothecin.

Figure1. In vitro cell viability of extract of *Tridax* and *Curcuma*:



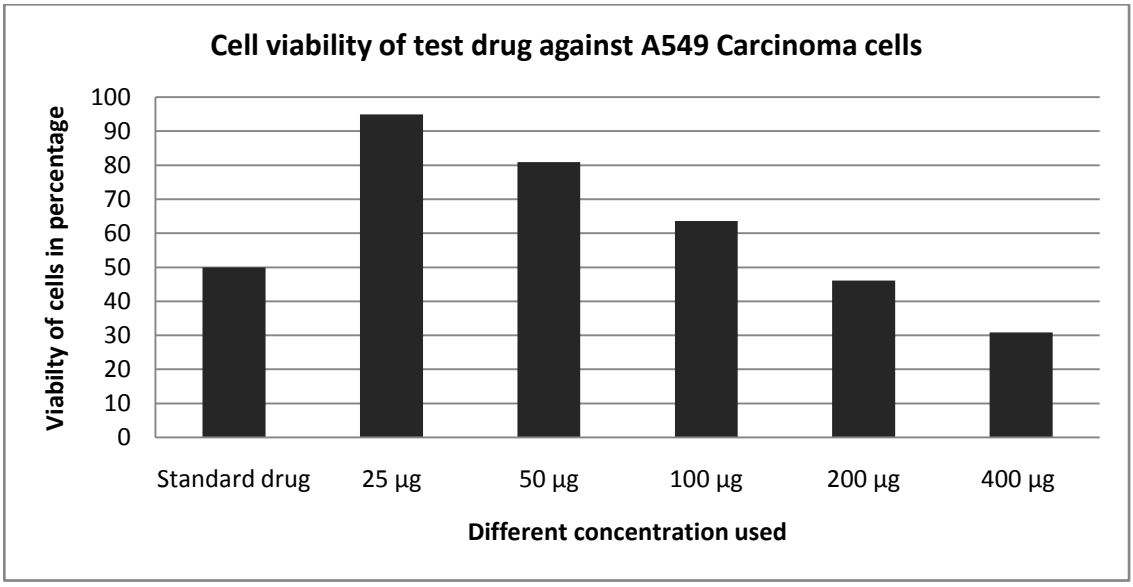
In this figure 1, the a) represents the negative control. The test drug in 25uG/mL shows in 94.9% of inhibition, 50uG/mL shows 80.8% of inhibition, 100Ug/ml shows 63.5% of inhibition, 200Ug/ml shows 46.05% of inhibition, 400Ug/ml shows 30.8% of inhibition.

Table 1: Concentration and viability of test drug

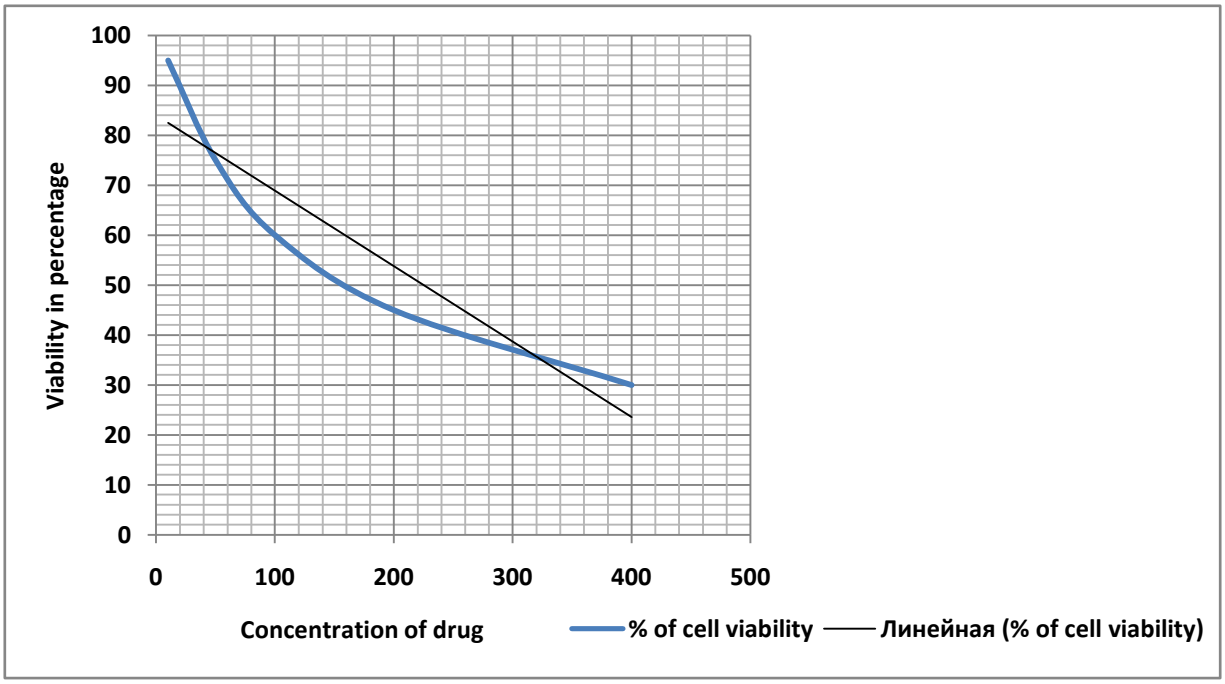
	BLANK	**UNTREADED**	**SD**	**25**	**50**	**100**	**200**	**400**
Reading 1	0.04	0.867	0.453	0.826	0.713	0.564	0.417	0.291
Reading 2	0.028	0.875	0.451	0.831	0.709	0.568	0.422	0.294
Mean	0.034	0.871	0.452	0.8285	0.711	0.566	0.4195	0.2925
Mean OD-Mean B		0.837	0.418	0.7945	0.677	0.532	0.3855	0.2585

STANDARD ERROR		0.005656854	0.001414214	0.003535534	0.002828427	0.002828427	0.003535534	0.002123120
STANDARD ERROR		0.004000604	0.001000151	0.002500378	0.002000302	0.002000302	0.002500378	0.001500227
Viability %		100	49.94026284	94.9223417	80.88410992	63.56033453	46.05734767	30.88410992

Figure 2. Percentage viability of test drug against a549 carcinoma cells.



IC50Value=175.20uG/mL



The direct Microscopic Observations of Drug Treated Images of A549 Cell linesby Inverted Biological Microscope after incubation of 24hours were enclosed in the separate folder with this report. Hence the IC50 Concentration of the combination of *Tridax* and *Curcuma*, against A549 Cell lines observed in the MTT Assay is 175.20Ug/ml.

CONCLUSION

Tridax procumbens, which is widely used in folklore medicine , has established its therapeutic uses with innumerable studies especially resulting in good anti cancer property . More work are to be done to further explore the wonderful prophylactic and therapeutic activities of the plant in the treatment of Cancer.

ACKNOWLEDGEMENT

We are thankful to our respected Madam. R. Sweety Nirmala, CEO, WSRPL, and beloved seniors. We express our sincere gratitude for affording facilities needed for accomplishment of this work.

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